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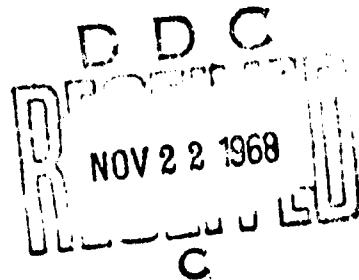
DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

PHYSICO-CHEMICAL INVESTIGATION OF THE MECHANISM OF
ENZYME INACTIVATION.
PART III. THERMODYNAMIC ANALYSIS OF STRUCTURAL AND
CHEMICAL CHANGES IN PROTEIN DURING RADIATION INACTIVATION.

L. Kh. Eydus
Institute of Biolog-
ical Physics,Moscow
Academy of Sciences,
USSR.

At the present time it has been established that the inactivation of
a number of enzymes in the presence of radiation effects takes place in two
stages, in the first of which long-lived latent damage develops which does
not affect the enzymatic function of the protein and is realized in visible
damage (inactivation) only in the second stage — in the presence of supple-
mentary non-radiation effects (1-4). One of the types of latent damage is
realized upon the action of oxygen on the anaerobically irradiated enzyme
(1-4); the other type, upon heating the irradiated protein in an aqueous
medium ("thermal" after-effect)(2-5).

As regards what structural and chemical changes take place in the
protein molecule during the individual stages of inactivation, at present
practically nothing is yet known. However, the revealed discreteness of the
various stages of radiation inactivation permits charting the course of the
study of these changes during each of the stages individually. In the pre-
sent report is proposed such a method of analysis, investigated with the
example of the radiation inactivation of myosin and pepsin — enzymes which
are quantitatively the most studied in the present connection (6). This
method consists of applying the method of thermodynamic analysis of protein
denaturing proposed by Eyring and Stearn (7, 8) to the thermal radiation
after-effect reaction. The information obtained as a result of such an anal-
ysis characterizes the structural and chemical changes in the irradiated
protein which take place in the second stage of inactivation — upon reali-
sation of the latent "thermal" damage. From the comparison of these changes
with those that take place upon thermal inactivation of intact protein con-
taining no latent damage, conclusions can also be drawn, in accordance with
the differential effect, concerning disturbances of the structure which are

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brought about in the first stage of the radiation effect upon the formation of latent damage.

This is depicted schematically in the illustration. Under number III in the illustration is designated the state of the "activated complex", from which the protein has already spontaneously passed over into the inactivated state. A whole set of various conformations of the protein structure fits the enzymatically active state; the transition from these to the activated complex requires the surmounting of energy barriers varying in magnitude and entropy changes. It is usually assumed that the activated complex in all these cases is the same; the difference consists only in the greater or lesser difficulty of its formation from one or another initial state of the protein.
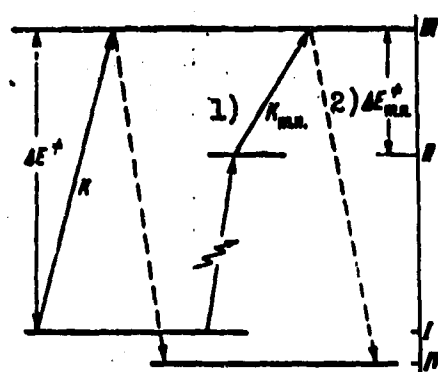
Legend: 1) $K_{t.a.}$

2) $\Delta E^{\neq}_{t.a.}$

Energy diagram of the two-stage inactivation of the molecules of enzymes in which latent damage of the "thermal" type (right section of the illustration) is created in the presence of radiation effects.

I — initial enzyme state; II — enzymatically active state with latent radiation damage; III — "activated complex"; IV — inactivated state. $K_{t.a.}$ and $\Delta E^{\neq}$ — rate constant and energy of activation of the thermal after-effect reaction; zigzag arrow — first stage of inactivation (creation of latent damage). On the left a diagram of the thermal inactivation of intact enzymes is given for comparison; K and $\Delta E^{\neq}$ — rate constant and energy of activation of this process.

We also will assume (see drawing) that molecules containing latent radiation damage pass over, when heated, into an activated complex state identical with the one that arises upon thermal inactivation of these proteins from any other enzymatically active conformations. Of course, it is

not possible to substantiate this hypothesis exactly. However, in the case
of myosin the results of our preceding work (9), in which it was shown that
for this object the activated complex is one and the same for inactivation
proceeding from different initial states of the intact protein, serves as a
serious argument in its favor.

A. Second Stage of Radiation Inactivation

The kinetic and thermodynamic characteristics of the inactivation of
intact enzymes and of the thermal after-effect reaction in the case of γ-
irradiation, which correspond to the experimental data given in (6), are
given in the table. Within the scope of the concepts of Eyring and Stearn
the conclusion should be drawn from analysis of this table that activation
in the thermal after-effect reaction consists of the rupture of one disul-
fide bond and approximately four hydrogen bonds. Of course it is not ruled
out that, along with the hydrogen bonds, not the disulfide bond but some
other covalent bond is ruptured here, and moreover within the limits of ac-
curacy of the experiments the whole series of $\Delta H_{t,a}^{\neq}$ and $\Delta S_{t,a}^{\neq}$ val-
ues obtained can be correlated. The assumption concerning the rupture of
only some hydrogen bonds is, however, clearly contradicted by these data;
in this case myosin, for example, would have $n_H/n_S = 2.25$. The arguments in
favor of the rupture of specifically the disulfide bond can be obtained
from examination of the results of radiation inactivation and in other as-
pects.

B. First Stage of Radiation Inactivation

From comparison of the structural changes taking place upon inactiva-
tion of intact proteins and proteins containing latent radiation damage it
should be concluded that the formation of latent damage (first stage of in-
activation) is accompanied by the rupture of $\sim$10-15 hydrogen bonds without
the rupture of covalent bridges. This conclusion correlates well with the
retention, at this stage of protein damage, of its enzyme activity, for the
maintenance of which the completeness of the molecule's tertiary structure
is apparently mainly responsible.

The physical process which can lead to simultaneous rupture of a num-
ber of hydrogen bonds in the irradiated protein molecule was pointed out in
their time by Platzman and Franck (10). It consists of the polarization of
the intramolecular medium as a result of the sudden formation of an electric
charge upon ionization of the protein molecule. The quantitative evaluation
of this effect, which was derived by the authors, agrees well with the val-
ues we obtained of the number of ruptured hydrogen bonds in the first stage
of inactivation. It should, however, be pointed out that, in the opinion of
the authors, one polarization effect only is insufficient for the inactiva-
tion of practically all the protein molecules except perhaps the ones smal-
lest in size (10, 11). This conclusion is connected with their denial of
the role of migration processes in the radiation damage of protein. Natural-
ly it is difficult to explain the great effectiveness of radiation inactiva-

Kinetic and Thermodynamic Characteristics of the Thermal Inactivation of Intact Enzymes and Thermal After-effect Reactions in -Irradiated Myosin and Pepsin Solutions

Enzyme	°C	lg K	$\Delta F^{\ddagger}$ kcal/mol	$\Delta H^{\ddagger}$ kcal/mol	$\Delta S^{\ddagger}$ kcal/degree·mol	$n_S = \frac{S^{\ddagger}}{12}$	$n_H = \frac{H^{\ddagger}-24}{4}$	$\frac{n_H}{n_S}$
Thermal Inactivation of Intact Enzymes								
Myosin	35	−4.02	25.9	97	237	19.8	18.3	0.92
	25	−6.35	26.1	97	238			
Pepsin	60	−4.5	26.5	90	190	15.8	16.5	1.04
Thermal After-effect in Irradiated Solutions								
Myosin	30	−3.5	22.7	38.4	52	4.3	3.6	0.84
Pepsin	56	−3.05	24.1	56.0	36.5	3.0	3.0	1.0

tion with an ion yield close to unity, as is observed in many enzymes, by the local "fusion" of 10-15 hydrogen bonds in any arbitrary section of the protein molecule. However, the situation can change in the presence of migration of the charge along the protein with its localization at the junction sites of the structure that are responsible for the configuration in the native state; for example, at the S—S bridges. In this case polarization would lead to deformation of this section, which could contribute to its subsequent rupture, in particular the heat-activated rupture of the disulfide bond /Note: The possibility of localization of the charge at the disulfide bond in irradiated protein molecules now has already been proved experimentally (12)./ during the second stage of inactivation.

CONCLUSIONS

1. A method for studying the structural and chemical changes during the individual stages of the radiation inactivation of enzymes is proposed, which consists of applying the Eyring-Stearn method of thermodynamic analysis of protein denaturing to the thermal radiation after-effect reaction.

2. It has been shown by this method that in the process of formation of latent damage in the myosin and pepsin molecules 10-15 hydrogen bonds are ruptured, but the covalent bonds are not ruptured; in the second stage of inactivation — upon realization of the latent damage — the rupture of one covalent bond (apparently the disulfide bond) and 3-4 hydrogen bonds takes place.

3. The hypothesis was expressed that the rupture of the hydrogen bonds in the first stage of inactivation takes place due to the Platzman-

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Franck polarization effect, but only after preliminary migration and localization of the charge at "weak" sites of the structure, particularly at the S-S bridges. "Fusion" of sections of the protein molecule in this region during the first stage of inactivation guarantees the possibility of carrying out the second stage.

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